PRELIMINARY GENETIC ANALYSIS OF CIRSIUM LONGISTYLUM (Long-styled thistle), A CANDIDATE THREATENED SPECIES

Prepared by:

Steven J. Brunsfeld and Calib T. Baldwin
Wildland Plant Ecogenetics Cooperative
University of Idaho

in cooperation with:

Montana Natural Heritage Program
State Library
1515 E. 6th Ave.
Helena, MT 59620

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EXECUTIVE SUMMARY

A genetic analysis of Cirsium longistylum (Long-styled thistle), a candidate threatened plant species, was initiated in 1993 to investigate hypothesized hybridization with C. hookerianum. The objective of the analysis was to determine if C. longistylum is a genetically unique entity, and to assess the importance of introgression in the evolution of the species. Enzyme electrophoresis was performed on 150 individuals from 12 populations of westcentral Montana. Cirsium longistylum was found to be genetically distinct from Montana populations of C. hookerianum and C. scariosum. Putative hybrids of C. longistylum were found to be most similar to "pure" C. longistylum plants, a finding consistent with either a hypothesis of hybridity or high natural variability within C. longistylum. A preliminary analysis of randomly amplified polymorphic DNA (RAPD) was initiated to gain better resolution of genetic relationships among C. longistylum, putative hybrids, and closely related Cirsium species. Twentyone samples representing C. longistylum, C. hookerianum, C. scariosum, and putative hybrids were analyzed, including four samples of C. hookerianum and C. scariosum from outside the study area in westcentral Montana. The RAPD data was congruent with electrophoretic data in identifying Cirsium longistylum as genetically distinct. Cirsium hookerianum and C. scariosum sampled outside the region were found to be genetically distant from samples of the same species in westcentral Montana. These data suggest that introgression historically may have occurred among all three species of the region. In order to document introgression using RAPD markers, a thorough understanding of the genetic composition of "pure" C. hookerianum and C. scariosum from outside the region is required. In addition, phylogenetic and additional morphological analyses are proposed as a basis for future conservation and management decisions.

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INTRODUCTION

Cirsium longistylum Moore & Frankton (Long-styled thistle) is a species endemic to four "island" mountain ranges of westcentral Montana, currently placed in Category 2 by the U.S. Fish and Wildlife Service as a candidate for federal listing as threatened (58 FR51144).

Cirsium longistylum is one of six closely related species of thistle distributed in the Rocky Mountains. Cirsium hookerianum Nutt. is primarily distributed in British Columbia and Alberta, but extends southward into westcentral and western Montana where it occurs sympatrically with C. longistylum in the Big Belt and Little Belt Mountain Ranges. Two additional species, Cirsium scariosum Nutt. and C. tweedyi (Rydb.) Petrak, approach the range of C. longistylum from the southwest. Cirsium scopulorum (Greene) Cockerell and C. eatonii (A. Gray) Robinson (Moore and Frankton 1965) occur south of Montana in the southern Rocky Mountains.

Survey and monitoring work has been conducted to characterize distribution and life history of Cirsium longistylum (Schassberger 1991, Schassberger and Achuff 1991, Roe 1992, Poole and Heidel 1993, Heidel 1994). At many sites, plants with bract characteristics deviating from the taxonomic circumscription of Moore and Frankton (1963) were noted. One of the three monitoring sites (Neihart) was interpreted as consisting primarily of Cirsium hookerianum, possibly with C. hookerianum x C. longistylum hybrids present (Cronquist pers. commun. as cited in Roe 1992). Monitoring work of the following year included an expanded morphological investigation (Poole and Heidel 1993). This investigation revealed that some populations contained plants with the diagnostic bract characteristics of both C. longistylum and C. hookerianum. Based on morphology it was concluded that Cirsium longistylum appeared to be a distinct species that hybridizes freely with C. hookerianum, producing swarms of morphologically variable individuals. This provisional interpretation warranted genetic documentation, and the present study was initiated to test the discreetness and persistence of a recognizable Cirsium longistylum genome.

METHODS

Sampling Procedures

Leaf samples of Cirsium longistylum were taken from seven study sites representing major population centers and a full range of morphological variation (Table 1). Collections were made of green leaf material on flowering stems, using the freshest available intact leaves, usually upper stem leaves. A minimum of two leaves were collected, sealed between damp paper towels in zip-loc plastic bags, labelled with an unique sequential number, stored on ice, and mailed in overnight mail to the University of Idaho. The bract category was recorded for each sampled individual as delimited in Poole and Heidel (1993).

At all sites of Cirsium longistylum, collections were made within a circular plot of 10 m radius, except for Russian Creek, with a 20 m radius. The sample areas corresponded to circular demographic monitoring plots at four of the seven sample sites (Heidel 1994). Sample size was a minimum of 20 plants per site, and included all flowering plants in the plot. Actual sample size ranged from 24 (Kings Hill #2-USFS) to 38 (S. Fk. Deadman Cr.) per site, though in most cases fewer than 15 plants were used per set for preliminary genetic analysis.

Six of the seven sample sets from the Little and Big Belt Mountain ranges represent discrete population at least 2 airmiles (3.2 km) apart. The Kings Hill pair of samples were collected from two adjoining segments of the population in monitoring plots from contrasting habitats.

Leaf size and condition varied between sites and within sites. The degree of inflorescence branching varied widely between plants, and upper stem leaf size was significantly smaller on the more highly-branched plants. Many stem leaves remained green through August in the exceptionally cool and moist season of 1993, but herbivory and rapid decay in storage markedly reduced usable leaf material.

Leaf samples of Cirsium hookerianum were collected at two sites over 30 miles (48 km) beyond the known range of Cirsium longistylum at Flesher Pass (Schassberger #464 !Cronquist) and Lewis and Clark Pass in the Blackfoot Range. Leaf samples of Cirsium scariosum were collected at one site over 50 miles (80 km) beyond the known range of Cirsium longistylum at Warren Pass in the Anaconda Range. These samples were collected in the first week of September when only basal rosette material retained living tissue.

DNA from herbarium specimens of Cirsium hookerianum from Pondera Co., MT (Hitchcock #18177) and Williams Lake, British Columbia (Calden #17953) and of C. scariosum from Nye Co., Nevada

Table 1. Sample Sites.

EO no.	Site name	Mt. range	County	Legal description	Bract category: l	lx	x	hx	h
Cirsium longistylum sites - all Little Belt Mt. sites are on Lewis and Clark Natl. Forest									
002	Kings Hill (#1-MTNHP)	Little Belts	Meagher	T.12N R.8E Sec. 2	9	10	11	5	1
002	Kings Hill (#2-USFS)	Little Belts	Meagher	T.12N R.8E Sec. 2	8	10	4	1	1
020	Russian Creek	Little Belts	Judith Basin	T.11N R.10E Sec. 11	25	4	1	-	-
008	Neihart	Little Belts	Cascade	T.14N R.7E Sec. 27	-	4	5	12	10
010	Moose Park	Little Belts	Cascade	T.13N R.8E Sec. 19	1	12	16	6	3
011	S. Fk. Deadman Cr.	Little Belts	Meagher	T.12N R.8E Sec. 24	22	7	4	-	-
006	Duck Creek Pass	Big Belts Helena Natl. Forest	Broadwater	T.9N R.4E Sec. 30	23	8	1	-	-
Cirsium hookerianum									
	Flesher Pass	Blackfoot		T.14N R.6W Sec. 3	Helena Natl. Forest				
	Lewis and Clark Pass	Blackfoot		T.16N R.7W Sec. 27	Helena Natl. Forest				
	Hitchcock (#18117) WSU	Pondera							
	Calder (#17953) WSU	Williams Lake, British Columbia CAN.							
Cirsium scariosum									
	Warren Pass	Anaconda		T.3N R.15W Sec. 22	Deerlodge Natl. Forest				
	Cronquist (#11040) WSU	Nye Co., Nevada							
	Baldwin (#380) ID	Klamath Co., Oregon			Winema Natl. Forest				

LX - *Cirsium longistylum* > *C. hookerianum*
X - *Cirsium longistylum* ↔ *C. hookerianum*
HX - *Cirsium longistylum* < *C. hookerianum*

(Cronquist #11040) and Klamath Co., Oregon (Baldwin #380) were utilized for the RAPD analysis.

Electrophoretic Analysis

Electrophoretic protocols employed were primarily those described in Brunsfeld et al. (1991) and Soltis et al. (1983). Leaf tissue was ground in Tris-HCL grinding buffer, absorbed onto filter paper wicks, loaded into starch gels, and electrophoresed for approximately six hours. In preliminary tests seventeen enzymes were stained: aconitase (ACN), alcohol dehydrogenase (ADH), acid phosphatase (APH), aspartate aminotransferase (AAT), esterase (EST), glutamate dehydrogenase (GDH), hexokinase (HK), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), malic enzyme (ME), menadione reductase (MNR), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6-PGD), shikimate dehydrogenase (SKDH), and triosephosphate isomerase (TPI). Of the enzymes tested, eleven (AAT, ADH, APH, IDH, LAP, MDH, PGI, PGM, 6-PGD,SKDH, and TPI) produced enzyme activity and were analyzed in all subsequent gel runs.

The genetic basis of enzyme banding patterns was inferred from observed segregation patterns in light of typical subunit structure and subcellular compartmentalization (Gottlieb 1981, Weeden and Wendel 1989). For enzymes with more than one locus (TPI), the isozymes were numbered sequentially, with the most anodal isozyme designated 1. Allozymes were labeled alphabetically starting with the fastest allozyme. Enzyme data were analyzed using BIOSYS-1 (Swofford and Selander 1981). Three measures of genetic diversity were calculated: mean number of alleles per locus (A), percentage of loci polymorphic (P), and mean expected heterozygosity (H_e). Genetic divergence among populations and species was analyzed using Nei's (1978) unbiased genetic identity measures calculated by BIOSYS-1, and a UPGMA cluster analysis of identity values was performed.

DNA Analysis

To gain better resolution of putative hybridization and genetic relationships among Cirsium species, we conducted a genetic analysis called "Randomly Amplified Polymorphic DNA" (RAPD), following the general methods of Williams et al. (1990) and Welsh and McClelland (1990). This technique involves amplifying numerous random portions of the plant genome using the polymerase chain reaction (PCR). In PCR, a heat-stable enzyme repeatedly replicates regions of the plant DNA, specifically where the enzyme is "primed" by small pieces of synthetic DNA (primers). The amplified DNA products are separated in a gel,

stained with a dye that fluoresces in ultraviolet light, and photographed.

Total DNA was extracted from each leaf sample using a modified CTAB method previously described (Brunsfeld et al. 1992). Purified DNA was next quantified and diluted to a uniform concentration (10 ng/ul). One hundred and twenty 10-mer primers of random sequence (Operon Technologies) were tested using a subset of Cirsium DNA samples.

RAPD products whose presence or absence was unambiguous were scored. Population samples were scored from at least three different amplification and electrophoretic runs, ensuring the repeatability of RAPD products. Phenetic segregation of populations based upon presence (1) and absence (0) of RAPD markers was conducted using Principal component analysis (PCA) (SAS Institute Inc., Cary, NC).

RESULTS

Enzyme Electrophoresis

Seven loci (AAT, APH, LAP, PGI, PGM, TPI-1, and TPI-2) were resolved for 150 individuals from twelve Cirsium populations from westcentral Montana. Data from five enzymes could not be used because of inconsistent staining or uninterpretable banding patterns. Data were sorted and analyzed two ways: by population/locality, and by species.

Population/Locality Analysis - For this analysis each locality was considered a single biological population and the tentative field identification of individuals was ignored. Table 2 identifies the names of the 12 populations or localities used in this analysis. Table 3 lists allele frequencies and the number of individuals sampled (N) at each locality. Sample sizes are small for some populations because of poor enzyme activity in many of the late season leaf samples.

Levels of genetic variation differ considerably among the 12 populations sampled (Table 4 and Appendix 1). Values of A ranged from 1.1 to 1.6; P varied from 14.3 to 42.9 percent; and H_e ranged from 0.006 to 0.110. Nei's (1978) unbiased genetic identity values were calculated for pairwise comparisons of all 12 populations (Table 5).

Table 2. Key to electrophoretic analysis by population.

Original pop. no.	Pop. no. on printout	Population name
FP	1	FLESHER PASS
DC	2	DUCK CREEK PASS
DM	3	DEADMAN CREEK
MP	4	MOOSE PARK
NH	5	NIEHART
RC	6	RUSSIAN CREEK
KH	7	KINGS HILL
SH	8	SHEEP CREEK
LC	9	LEWIS & CLARK
AC	10	ALICE CREEK
WP	11	WARREN PASS
SL	12	SLAUGHTERHOUSE

Table 3. Allele frequencies by locus and population.

	Population											
Locus	1	2	3	4	5	6	7	8	9	10	11	12
PGI-1												
(N)	21	18	5	5	5	4	4	3	11	8	9	14
A	1.000	.917	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	.000	.083	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
TPI-1												
(N)	30	18	5	5	5	17	4	3	14	10	12	24
A	.033	.056	.100	.100	.000	.147	.000	.000	.071	.050	.042	.000
B	.933	.944	.900	.900	1.000	.794	1.000	1.000	.929	.950	.958	1.000
C	.033	.000	.000	.000	.000	.059	.000	.000	.000	.000	.000	.000
TPI-2												
(N)	15	18	5	5	5	17	4	3	11	10	13	24
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LAP-1												
(N)	13	18	5	2	5	22	4	3	10	7	5	10
A	.000	.000	.000	.000	.000	.023	.000	.000	.000	.000	.000	.000
B	.731	.556	.500	.750	.900	.614	.125	.333	.950	.857	1.000	1.000
C	.269	.444	.500	.000	.100	.364	.875	.667	.050	.143	.000	.000
D	.000	.000	.000	.250	.000	.000	.000	.000	.000	.000	.000	.000
APH-1												
(N)	20	18	5	5	5	17	4	3	11	10	13	10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
AAT-1												
(N)	18	18	5	5	5	17	4	3	7	6	8	14
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM-1												
(N)	25	13	5	5	5	13	4	3	13	11	12	24
A	.080	.000	.000	.000	.000	.000	.000	.000	.346	.182	.042	.000
B	.920	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.654	.818	.958	.979
C	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.021

able 4. Genetic variability by populations (standard errors in parentheses).

Population	Mean sample size per Locus	Mean no. of alleles per locus	Percentage of loci polymorphic*	Mean heterozygosity Direct-count	Mean heterozygosity HdyWbg expected**
1. FLESHER PASS	20.3 (2.2)	1.6 (0.3)	42.9	.075 (.037)	.098 (.058)
2. DUCK CREEK PASS	17.3 (0.7)	1.4 (0.2)	42.9	.087 (.048)	.110 (.071)
3. DEADMAN CREEK	5.0 (0.0)	1.3 (0.2)	28.6	.114 (.086)	.108 (.080)
4. MOOSE PARK	4.6 (0.4)	1.3 (0.2)	28.6	.100 (.072)	.100 (.072)
5. NIEHART	5.0 (0.0)	1.1 (0.1)	14.3	.029 (.029)	.029 (.029)
6. RUSSIAN CREEK	15.3 (2.1)	1.6 (0.4)	28.6	.089 (.058)	.122 (.081)
7. KINGS HILL	4.0 (0.0)	1.1 (0.1)	14.3	.036 (.036)	.036 (.036)
8. SHEEP CREEK	3.0 (0.0)	1.1 (0.1)	14.3	.095 (.095)	.076 (.076)
9. LEWIS & CLARK	11.0 (0.8)	1.4 (0.2)	42.9	.046 (.023)	.101 (.065)
10. ALICE CREEK	8.9 (0.7)	1.4 (0.2)	42.9	.040 (.027)	.096 (.052)
11. WARREN PASS	10.3 (1.1)	1.3 (0.2)	28.6	.024 (.015)	.024 (.015)
12. SLAUGHTERHOUSE	17.1 (2.5)	1.1 (0.1)	14.3	.006 (.006)	.006 (.006)

* A locus is considered polymorphic if more than one allele was detected.

** Unbiased estimate (see Nei, 1978).

able 5. Nei (1978) unbiased genetic identity by population.

Population	1	2	3	4	5	6	7	8
1 FLESHER PASS	*****							
2 DUCK CREEK PASS	.996	*****						
3 DEADMAN CREEK	.998	1.000	*****					
4 MOOSE PARK	1.000	.988	.988	*****				
5 NIEHART	.998	.984	.982	1.000	*****			
6 RUSSIAN CREEK	.998	.999	1.000	.995	.988	*****		
7 KINGS HILL	.947	.975	.986	.919	.915	.961	*****	
8 SHEEP CREEK	.982	1.000	1.000	.964	.960	.990	1.000	*****
9 LEWIS & CLARK	.985	.959	.957	.986	.985	.966	.882	.930
10 ALICE CREEK	1.000	.984	.983	1.000	.999	.988	.918	.962
11 WARREN PASS	.992	.972	.968	1.000	1.000	.978	.890	.940
12 SLAUGHTERHOUSE	.991	.972	.968	1.000	1.000	.977	.891	.941

Population	9	10	11	12
9 LEWIS & CLARK	*****			
10 ALICE CREEK	1.000	*****		
11 WARREN PASS	.989	.998	*****	
12 SLAUGHTERHOUSE	.985	.996	1.000	*****

The UPGMA cluster analysis of the Nei identity values is shown in Figure 1. Populations cluster into two major groups with an overall similarity of 0.96; one group contains C. longistylum (L) and putative hybrids (LX, X, Table 1), the second group contains C. scariosum (S), C. hookerianum (H), and putative hybrids. The three pure or largely pure populations of C. longistylum (Duck Creek Pass, Deadman Creek, Russian Creek) form a distinct subcluster with a similarity of approximately 0.98. The remaining two populations in the group (Kings Hill [USFS] and Sheep Creek) are represented by small samples consisting of a mixture of L, LX, X, and a single H.

In the second large cluster, one "pure" C. hookerianum population (Lewis and Clark) is the most distinct, and two other "pure" C. hookerianum populations (Flesher Pass and Alice Creek) cluster a small sample of hybrids (Moose Park). In addition, two "pure" C. scariosum populations cluster with another small group of putative hybrids. No "pure" C. longistylum individuals were part of the sample in any of the 6 populations in this cluster.

Species Analysis - In this analysis individuals were grouped by their field identification, i.e. L, H, S, LX, and X. To increase sample sizes individuals for different populations were grouped together. Table 6 identifies the names of the species used in this analysis. Table 7 lists allele frequencies and the number of individuals sampled (N) of each species or hybrid class. Genetic variation statistics for each group are presented in Table 8 and Appendix II. Values of A ranged from 1.3 to 1.7; P varied from 28.6 to 57.1 percent; and H_e ranged from 0.019 to 0.123. Nei's (1978) unbiased genetic identity values were calculated for pairwise comparisons of all 5 species or hybrid groups (Table 9). A UPGMA cluster analysis of this data matrix (Figure 2) has two major clusters: one containing C. longistylum individuals and those considered hybrids of C. longistylum and C. hookerianum; and a second cluster of "pure" C. hookerianum and C. scariosum, which have similarity of 0.99 based on these isozyme loci.

DNA Analysis

A preliminary analysis of twentyone Cirsium samples was conducted. These included: 6 "pure" C. longistylum, 5 C. hookerianum from Montana, 1 C. hookerianum from British Columbia, 4 C. scariosum from Montana, 2 C. scariosum from Nevada and Oregon, and three hybrids (HX, LX, X). Fortyseven RAPD loci were scored for each sample. The first 3 principal components accounted for 47% of the variance in the data set (Appendix III). Figures 3 and 4 display the distribution of samples in principal component space.

Table 6. Key to electrophoretic analysis by species.

Original pop. no.	Pop. no. on printout	Population name
SC	1	SCARIOSUM
HO	2	HOOKERIANUM
X	3	HOOKxLONG (X)
LX	4	LONGxHOOK (LX)
LO	5	LONGISTYLUM

Table 7. Allele frequency by locus and species.

	Population				
Locus	1	2	3	4	5
PGI-1					
(N)	23	44	5	12	14
A	1.000	1.000	.900	.958	.964
B	.000	.000	.100	.042	.036
TPI-1					
(N)	36	58	5	12	30
A	.042	.043	.000	.083	.133
B	.958	.940	1.000	.917	.833
C	.000	.017	.000	.000	.033
TPI-2					
(N)	37	40	5	12	36
A	1.000	.988	1.000	1.000	1.000
B	.000	.013	.000	.000	.000
LAP-1					
(N)	15	35	4	11	35
A	.000	.000	.000	.000	.014
B	1.000	.814	.375	.545	.600
C	.000	.186	.625	.409	.386
D	.000	.000	.000	.045	.000
APH-1					
(N)	23	45	5	9	36
A	1.000	1.000	1.000	1.000	1.000
AAT-1					
(N)	22	35	5	11	35
A	1.000	1.000	1.000	1.000	1.000
PGM-1					
(N)	36	48	5	11	31
A	.014	.188	.000	.000	.000
B	.972	.813	1.000	1.000	1.000
C	.014	.000	.000	.000	.000

Table 8. Genetic variability by species (standard errors in parentheses).

Population	Mean sample size per Locus	Mean no. of alleles per locus	Percentage of loci polymorphic*	Mean heterozygosity	
				Direct-count	HdyWbg expected**
1. SCARIOSUM	27.4 (3.3)	1.4 (0.3)	28.6	.020 (.013)	.019 (.013)
2. HOOKERIANUM	43.6 (3.0)	1.7 (0.3)	57.1	.065 (.028)	.108 (.054)
3. HOOKxLONG(X)	4.9 (0.1)	1.3 (0.2)	28.6	.064 (.042)	.105 (.077)
4. LONGxHOOK(LX)	11.1 (0.4)	1.6 (0.3)	42.9	.140 (.101)	.114 (.078)
5. LONGISTYLUM	31.0 (3.0)	1.7 (0.4)	42.9	.098 (.055)	.123 (.074)

* A locus is considered polymorphic if more than one allele was detected.

** Unbiased estimate (see Nei, 1978).

Table 9. Nei (1978) unbiased genetic identity by species.

Population	1	2	3	4	5
1 SCARIOSUM	*****				
2 HOOKERIANUM	.008	*****			
3 HOOKxLONG(X)	.055	.031	*****		
4 LONGxHOOK(LX)	.026	.013	.000	*****	
5 LONGISTYLUM	.023	.013	.005	.000	*****

Figure 1. UPGMA cluster analysis of electrophoretic data by population, using Nei (1978) unbiased genetic identity.

```
.93       .95       .97       .98      1.00
+----+----+----+----+----+----+----+----+
                                       * FLESHER PASS (Hookerianum)
                                       *
                                     *** MOOSE PARK (3X:2LX)
                                     * *
                                     * * ALICE CREEK (Hookerianum)
                                     *
                                ******
                                *    * * NIEHART (2X:3H)
                                *    * *
               ******************    *** WARREN PASS (Scariosum)
               *                *      *
               *                *      * SLAUGHTERHOUSE (Scariosum)
               *                *
               *                ******** LEWIS & CLARK (Hookerianum)
               *
               *                       * DUCK CREEK PASS (13L:4LX:1X)
               *                       *
               *              ********** DEADMAN CREEK (3L:2LX)
               *              *        *
               ****************        * RUSSIAN CREEK (Longistylum)
                              *
                              *        * KINGS HILL (2L:1LX:1X)
                              **********
                                       * SHEEP CREEK (1L:1LX:1H)
+----+----+----+----+----+----+----+----+
.93       .95       .97       .98      1.00
                 Similarity
```

Figure 2. UPGMA cluster analysis of electrophoretic data by species, using Nei (1978) unbiased genetic identity.

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.93       .95       .97       .98      1.00
+----+----+----+----+----+----+----+----+
                                   ****** SCARIOSUM
                                   *
                         ***********
                         *         ****** HOOKERIANUM
                         *
                         *
                         *               * HOOKxLONG(X)
                         *               *
                         *             * *
                         *             * * LONGxHOOK(LX)
                         ***************
                                       *
                                       * * LONGISTYLUM
+----+----+----+----+----+----+----+----+
.93       .95       .97       .98      1.00
                 Similarity
```

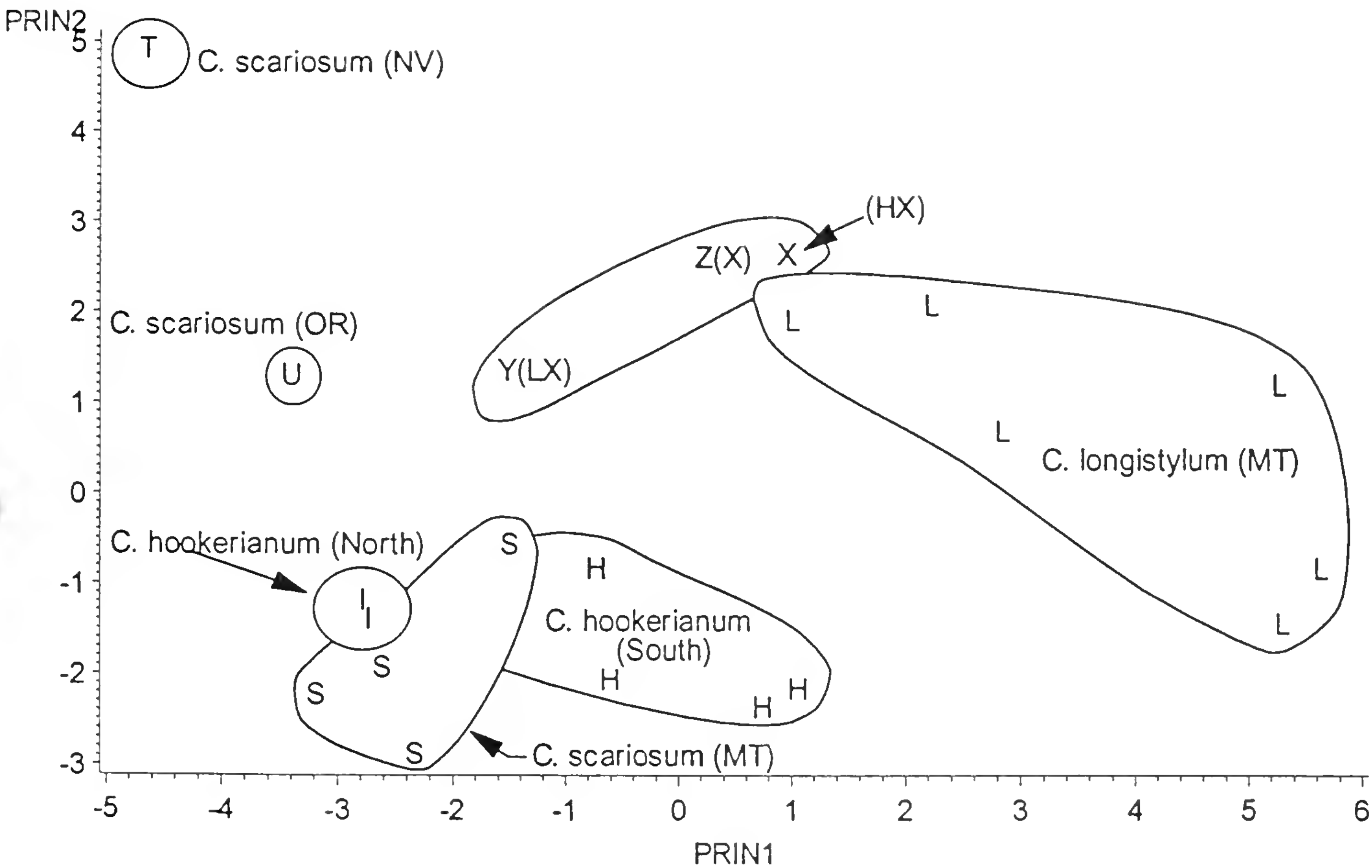

Figure 3. PCA of RAPD data from C. longistylum (L) EO number 020, 011 & 006, C. hookerianum from southwestern Montana (H), C. hookerianum from northern Montana and British Columbia (I), C. scariosum from southwestern Montana (S), C. scariosum from Nevada (T), C. scariosum from Oregon (U), and putative hybrids HX (X), LX (Y) and X (Z).

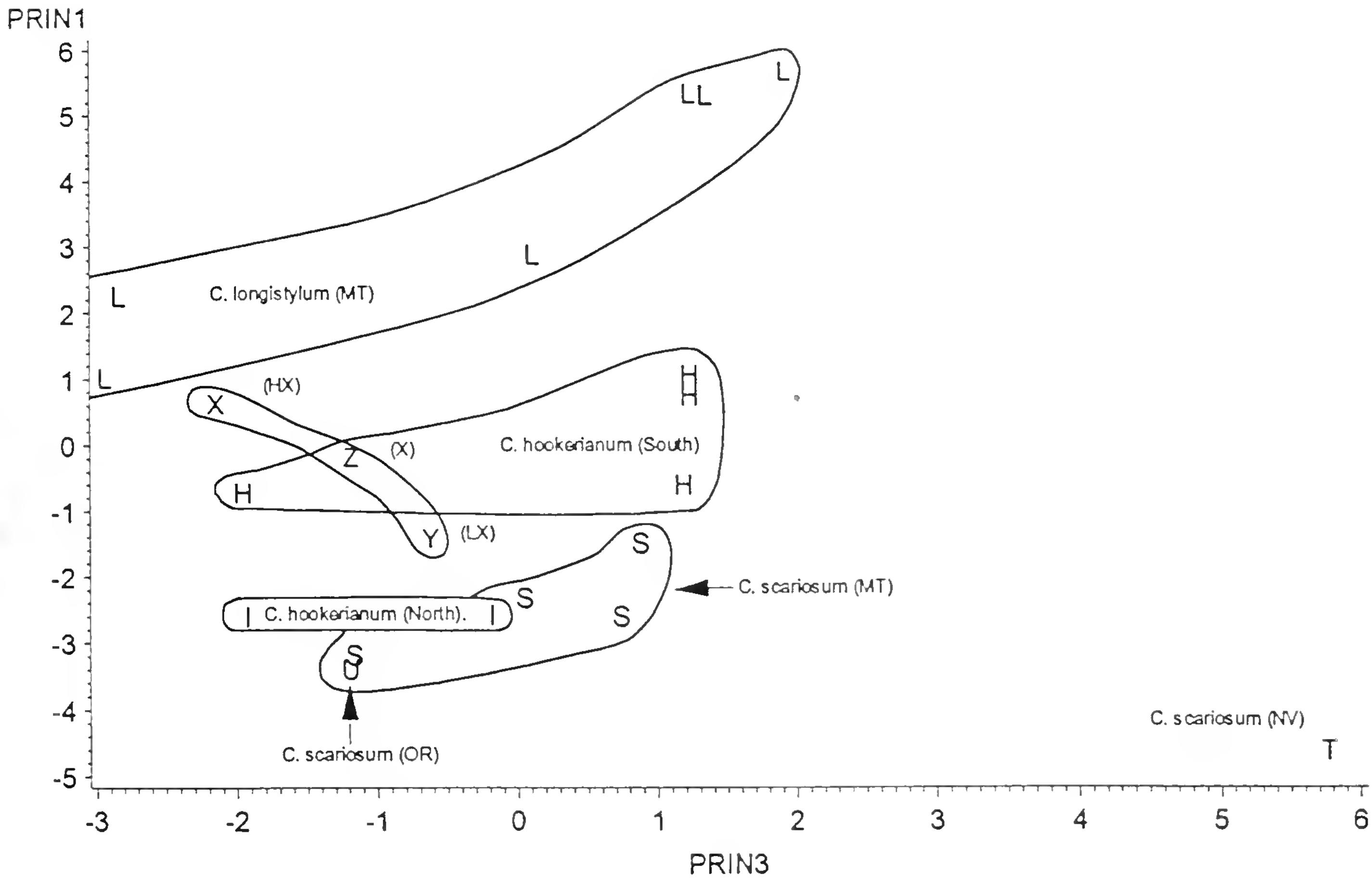

Figure 4. PCA of RAPD data from C. longistylum (L) EO number 020, 011 & 006, C. hookerianum from southwestern Montana (H), C. hookerianum from northern Montana and British Columbia (I), C. scariosum from southwestern Montana (S), C. scariosum from Nevada (T), C. scariosum from Oregon (U), and putative hybrids HX (X), LX (Y) and X (Z).

Cirsium longistylum is strongly separated from both C. scariosum and C. hookerianum. Cirsium scariosum from Montana is distinct from the samples from Nevada and Oregon. Similarly, C. hookerianum samples from British Columbia and northern Montana separate from westcentral Montana samples. Hybrids as a group are scarcely distinguishable from C. longistylum and C. hookerianum.

DISCUSSION

Enzyme Electrophoresis Analysis - The Cirsium samples we analyzed contain levels of enzyme variability that would be expected, based on data from other species with similar life history characteristics (Hamrick & Godt 1989). Whether data is sorted and analyzed by taxonomic classification or population/locality, similar results are obtained. Plants identified in the field as Cirsium longistylum are genetically distinct from plants identified as C. hookerianum and C. scariosum. Indeed, of the three species sampled from westcentral Montana, C. longistylum appears to be the most genetically distinct. Three populations that are considered to be pure or nearly pure C. longistylum cluster together in our analysis, and are very distinct from populations of C. hookerianum and C. scariosum, which appear more closely related. All plants classified as hybrids of C. longistylum cluster with pure C. longistylum, whereas populations composed of a mixture of parents and putative hybrids are heterogeneous - two cluster with C. longistylum, one clusters with C. hookerianum, and one clusters with C. scariosum.

While these results are consistent with the hypothesis that hybridization is occurring between C. longistylum and another species, enzyme electrophoresis does not provide enough diagnostic genetic markers to rule out alternative explanations.

DNA Analysis - Preliminary results of the analysis of RAPD data are congruent with electrophoretic results, and also provide additional insights into Cirsium genetics. Plants identified as Cirsium longistylum are separable from all others, but exhibit more variability than other species within westcentral Montana. Several of the C. longistylum samples are among the most genetically distinct plants that we analyzed, suggesting that C. longistylum represents a well-differentiated evolutionary lineage. Whether the variability within C. longistylum is attributable to hybridization requires more detailed investigation.

Two widely separated northern samples of C. hookerianum, obtained from herbarium specimens, are similar to each other, but differentiated from westcentral Montana samples. Based on morphology, Cronquist (1964) considered northern C. hookerianum

populations to be the purest form of the species. A better genetic understanding of "pure" northern _C. hookerianum_ is needed to interpret variability in westcentral Montana _C. hookerianum_ and _C. longistylum_. Distant samples of _C. scariosum_ from Nevada and Oregon are also genetically distinct from westcentral Montana _C. scariosum_. Furthermore, the relative genetic similarity of _C. scariosum_ and _C. hookerianum_ from westcentral Montana raises the question of whether introgression has affected all of the species in this region.

In the PCA analysis of RAPD data, putative hybrid plants are placed near or intermediate between _C. longistylum_, _C. hookerianum_, and _C. scariosum_ plants, consistent with the hypothesis that they arose by hybridization. However, until the genetic composition of "pure" _C. longistylum_, _C. hookerianum_, and _C. scariosum_ is understood, through rangewide sampling of the species, it will not be possible to distinguish between natural variability in each species and variability induced by hybridization/ introgression.

Hypothesized Recent Evolutionary History of _Cirsium longistylum_

The evolutionary history of _Cirsium_ species in Montana can be hypothesized in light of existing genetic, morphologic, and geographic information. Our preliminary DNA analysis suggests that _Cirsium longistylum_, _C. hookerianum_, and _C. scariosum_ are well-differentiated entities in the allopatric parts of their ranges, apparently representing separate evolutionary lineages. During glacial times, the northern species of the group, _C. hookerianum_, was likely forced southward into sympatry with _C. longistylum_ in westcentral and southern Montana. _Cirsium scariosum_ also colonized the region, most likely from its principal range to the southwest. Gene flow via wind-dispersed seed and nondiscriminating pollinators probably occurred among many populations in westcentral Montana, resulting in regional introgression of the three species. _Cirsium longistylum_ genes have introgressed into _C. hookerianum_ and _C. scariosum_ producing distinct westcentral Montana races of these species. Because genes from all three species are likely widespread throughout westcentral Montana, distinguishing between introgression and natural variation within each species is not possible without understanding genetic patterns and variability outside of westcentral Montana.

A phylogenetic analysis of all _Cirsium_ species in the region is needed to test the evolutionary hypotheses presented above. Knowledge of phylogenetic relationships and processes in _Cirsium_ would allow managers to judge: the biological significance of _C. longistylum_, whether human activity or natural processes have produced the current biological situation, and whether any further management action is warranted.

Future Work

To gain a complete understanding of *C. longistylum*, the following additional work is needed:

1) A restriction site analysis of chloroplast DNA should be performed on *C. longistylum* and all closely related species. The phylogeny derived from these data will be the cornerstone of our understanding of the genetic significance of the *C. longistylum* lineage and the evolutionary events that occurred in Montana.

2) Gain a better understanding hybridization and introgression in Montana by obtaining essential information on the variability of *C. hookerianum* and *C. scariosum* throughout their geographic range.

3) Study the morphology of genetically pure *C. longistylum*, *C. hookerianum*, and *C. scariosum* so that botanists and managers can more easily interpret plants encountered in the field.

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APPENDIX I

Allele frequencies and genetic variability measures of electrophoretic data by population.

Population: FLESHER PASS (FP)

	Locus and sample size						
Allele	PGI-1 21	TPI-1 30	TPI-2 15	LAP-1 13	APH-1 20	AAT-1 18	PGM-1 25
A	1.000	.033	1.000	.000	1.000	1.000	.080
B	.000	.933	.000	.731	.000	.000	.920
C	.000	.033	.000	.269	.000	.000	.000
H	.000	.127	.000	.393	.000	.000	.147
H(unb)	.000	.129	.000	.409	.000	.000	.150
H(D.C.)	.000	.133	.000	.231	.000	.000	.160

Mean heterozygosity per locus (biased estimate) = .095 (S.E. .055)
Mean heterozygosity per locus (unbiased estimate) = .098 (S.E. .058)
Mean heterozygosity per locus (direct-count estimate) = .075 (S.E. .037)
Mean number of alleles per locus = 1.57 (S.E. .30)
Percentage of loci polymorphic (0.95 criterion) = 42.86
Percentage of loci polymorphic (0.99 criterion) = 42.86
Percentage of loci polymorphic (no criterion) = 42.86

Population: DUCK CREEK PASS (DC)

	Locus and sample size						
Allele	PGI-1 18	TPI-1 18	TPI-2 18	LAP-1 18	APH-1 18	AAT-1 18	PGM-1 13
A	.917	.056	1.000	.000	1.000	1.000	.000
B	.083	.944	.000	.556	.000	.000	1.000
C	.000	.000	.000	.444	.000	.000	.000
H	.153	.105	.000	.494	.000	.000	.000
H(unb)	.157	.108	.000	.508	.000	.000	.000
H(D.C.)	.167	.111	.000	.333	.000	.000	.000

Mean heterozygosity per locus (biased estimate) = .107 (S.E. .069)
Mean heterozygosity per locus (unbiased estimate) = .110 (S.E. .071)
Mean heterozygosity per locus (direct-count estimate) = .087 (S.E. .048)
Mean number of alleles per locus = 1.43 (S.E. .20)
Percentage of loci polymorphic (0.95 criterion) = 42.86
Percentage of loci polymorphic (0.99 criterion) = 42.86
Percentage of loci polymorphic (no criterion) = 42.86

Population: DEADMAN CREEK (DM)

Allele	PGI-1 5	TPI-1 5	TPI-2 5	LAP-1 5	APH-1 5	AAT-1 5	PGM-1 5
	Locus and sample size						
A	1.000	.100	1.000	.000	1.000	1.000	.000
B	.000	.900	.000	.500	.000	.000	1.000
C	.000	.000	.000	.500	.000	.000	.000
H	.000	.180	.000	.500	.000	.000	.000
H(unb)	.000	.200	.000	.556	.000	.000	.000
H(D.C.)	.000	.200	.000	.600	.000	.000	.000

Mean heterozygosity per locus (biased estimate) = .097 (S.E. .072)
Mean heterozygosity per locus (unbiased estimate) = .108 (S.E. .080)
Mean heterozygosity per locus (direct-count estimate) = .114 (S.E. .086)
Mean number of alleles per locus = 1.29 (S.E. .18)
Percentage of loci polymorphic (0.95 criterion) = 28.57
Percentage of loci polymorphic (0.99 criterion) = 28.57
Percentage of loci polymorphic (no criterion) = 28.57

Population: MOOSE PARK (MP)

Allele	PGI-1 5	TPI-1 5	TPI-2 5	LAP-1 2	APH-1 5	AAT-1 5	PGM-1 5
	Locus and sample size						
A	1.000	.100	1.000	.000	1.000	1.000	.000
B	.000	.900	.000	.750	.000	.000	1.000
C	.000	.000	.000	.000	.000	.000	.000
D	.000	.000	.000	.250	.000	.000	.000
H	.000	.180	.000	.375	.000	.000	.000
H(unb)	.000	.200	.000	.500	.000	.000	.000
H(D.C.)	.000	.200	.000	.500	.000	.000	.000

Mean heterozygosity per locus (biased estimate) = .079 (S.E. .055)
Mean heterozygosity per locus (unbiased estimate) = .100 (S.E. .072)
Mean heterozygosity per locus (direct-count estimate) = .100 (S.E. .072)
Mean number of alleles per locus = 1.29 (S.E. .18)
Percentage of loci polymorphic (0.95 criterion) = 28.57
Percentage of loci polymorphic (0.99 criterion) = 28.57
Percentage of loci polymorphic (no criterion) = 28.57

Population: NIEHART (NH)

	Locus and sample size						
	PGI-1	TPI-1	TPI-2	LAP-1	APH-1	AAT-1	PGM-1
Allele	5	5	5	5	5	5	5
A	1.000	.000	1.000	.000	1.000	1.000	.000
B	.000	1.000	.000	.900	.000	.000	1.000
C	.000	.000	.000	.100	.000	.000	.000
H	.000	.000	.000	.180	.000	.000	.000
H(unb)	.000	.000	.000	.200	.000	.000	.000
H(D.C.)	.000	.000	.000	.200	.000	.000	.000

Mean heterozygosity per locus (biased estimate) = .026 (S.E. .026)
Mean heterozygosity per locus (unbiased estimate) = .029 (S.E. .029)
Mean heterozygosity per locus (direct-count estimate) = .029 (S.E. .029)
Mean number of alleles per locus = 1.14 (S.E. .14)
Percentage of loci polymorphic (0.95 criterion) = 14.29
Percentage of loci polymorphic (0.99 criterion) = 14.29
Percentage of loci polymorphic (no criterion) = 14.29

Population: RUSSIAN CREEK (RC)

	Locus and sample size						
	PGI-1	TPI-1	TPI-2	LAP-1	APH-1	AAT-1	PGM-1
Allele	4	17	17	22	17	17	13
A	1.000	.147	1.000	.023	1.000	1.000	.000
B	.000	.794	.000	.614	.000	.000	1.000
C	.000	.059	.000	.364	.000	.000	.000
H	.000	.344	.000	.491	.000	.000	.000
H(unb)	.000	.355	.000	.502	.000	.000	.000
H(D.C.)	.000	.353	.000	.273	.000	.000	.000

Mean heterozygosity per locus (biased estimate) = .119 (S.E. .079)
Mean heterozygosity per locus (unbiased estimate) = .122 (S.E. .081)
Mean heterozygosity per locus (direct-count estimate) = .089 (S.E. .058)
Mean number of alleles per locus = 1.57 (S.E. .37)
Percentage of loci polymorphic (0.95 criterion) = 28.57
Percentage of loci polymorphic (0.99 criterion) = 28.57
Percentage of loci polymorphic (no criterion) = 28.57

Population: KINGS HILL (KH)

	Locus and sample size						
	PGI-1	TPI-1	TPI-2	LAP-1	APH-1	AAT-1	PGM-1
Allele	4	4	4	4	4	4	4
A	1.000	.000	1.000	.000	1.000	1.000	.000
B	.000	1.000	.000	.125	.000	.000	1.000
C	.000	.000	.000	.875	.000	.000	.000
H	.000	.000	.000	.219	.000	.000	.000
H(unb)	.000	.000	.000	.250	.000	.000	.000
H(D.C.)	.000	.000	.000	.250	.000	.000	.000

Mean heterozygosity per locus (biased estimate) = .031 (S.E. .031)
Mean heterozygosity per locus (unbiased estimate) = .036 (S.E. .036)
Mean heterozygosity per locus (direct-count estimate) = .036 (S.E. .036)
Mean number of alleles per locus = 1.14 (S.E. .14)
Percentage of loci polymorphic (0.95 criterion) = 14.29
Percentage of loci polymorphic (0.99 criterion) = 14.29
Percentage of loci polymorphic (no criterion) = 14.29

Population: SHEEP CREEK (SH)

	Locus and sample size						
	PGI-1	TPI-1	TPI-2	LAP-1	APH-1	AAT-1	PGM-1
Allele	3	3	3	3	3	3	3
A	1.000	.000	1.000	.000	1.000	1.000	.000
B	.000	1.000	.000	.333	.000	.000	1.000
C	.000	.000	.000	.667	.000	.000	.000
H	.000	.000	.000	.444	.000	.000	.000
H(unb)	.000	.000	.000	.533	.000	.000	.000
H(D.C.)	.000	.000	.000	.667	.000	.000	.000

Mean heterozygosity per locus (biased estimate) = .063 (S.E. .063)
Mean heterozygosity per locus (unbiased estimate) = .076 (S.E. .076)
Mean heterozygosity per locus (direct-count estimate) = .095 (S.E. .095)
Mean number of alleles per locus = 1.14 (S.E. .14)
Percentage of loci polymorphic (0.95 criterion) = 14.29
Percentage of loci polymorphic (0.99 criterion) = 14.29
Percentage of loci polymorphic (no criterion) = 14.29

Population: LEWIS & CLARK (LC)

	Locus and sample size						
	PGI-1	TPI-1	TPI-2	LAP-1	APH-1	AAT-1	PGM-1
Allele	11	14	11	10	11	7	13
A	1.000	.071	1.000	.000	1.000	1.000	.346
B	.000	.929	.000	.950	.000	.000	.654
C	.000	.000	.000	.050	.000	.000	.000
H	.000	.133	.000	.095	.000	.000	.453
H(unb)	.000	.138	.000	.100	.000	.000	.471
H(D.C.)	.000	.143	.000	.100	.000	.000	.077

Mean heterozygosity per locus (biased estimate) = .097 (S.E. .063)
Mean heterozygosity per locus (unbiased estimate) = .101 (S.E. .065)
Mean heterozygosity per locus (direct-count estimate) = .046 (S.E. .023)
Mean number of alleles per locus = 1.43 (S.E. .20)
Percentage of loci polymorphic (0.95 criterion) = 42.86
Percentage of loci polymorphic (0.99 criterion) = 42.86
Percentage of loci polymorphic (no criterion) = 42.86

Population: ALICE CREEK (AC)

	Locus and sample size						
	PGI-1	TPI-1	TPI-2	LAP-1	APH-1	AAT-1	PGM-1
Allele	8	10	10	7	10	6	11
A	1.000	.050	1.000	.000	1.000	1.000	.182
B	.000	.950	.000	.857	.000	.000	.818
C	.000	.000	.000	.143	.000	.000	.000
H	.000	.095	.000	.245	.000	.000	.298
H(unb)	.000	.100	.000	.264	.000	.000	.312
H(D.C.)	.000	.100	.000	.000	.000	.000	.182

Mean heterozygosity per locus (biased estimate) = .091 (S.E. .049)
Mean heterozygosity per locus (unbiased estimate) = .096 (S.E. .052)
Mean heterozygosity per locus (direct-count estimate) = .040 (S.E. .027)
Mean number of alleles per locus = 1.43 (S.E. .20)
Percentage of loci polymorphic (0.95 criterion) = 42.86
Percentage of loci polymorphic (0.99 criterion) = 42.86
Percentage of loci polymorphic (no criterion) = 42.86

Population: WARREN PASS (WP)

	Locus and sample size						
Allele	PGI-1 9	TPI-1 12	TPI-2 13	LAP-1 5	APH-1 13	AAT-1 8	PGM-1 12
A	1.000	.042	1.000	.000	1.000	1.000	.042
B	.000	.958	.000	1.000	.000	.000	.958
H	.000	.080	.000	.000	.000	.000	.080
H(unb)	.000	.083	.000	.000	.000	.000	.083
H(D.C.)	.000	.083	.000	.000	.000	.000	.083

Mean heterozygosity per locus (biased estimate) = .023 (S.E. .015)
Mean heterozygosity per locus (unbiased estimate) = .024 (S.E. .015)
Mean heterozygosity per locus (direct-count estimate) = .024 (S.E. .015)
Mean number of alleles per locus = 1.29 (S.E. .18)
Percentage of loci polymorphic (0.95 criterion) = .00
Percentage of loci polymorphic (0.99 criterion) = 28.57
Percentage of loci polymorphic (no criterion) = 28.57

Population: SLAUGHTERHOUSE (SL)

	Locus and sample size						
Allele	PGI-1 14	TPI-1 24	TPI-2 24	LAP-1 10	APH-1 10	AAT-1 14	PGM-1 24
A	1.000	.000	1.000	.000	1.000	1.000	.000
B	.000	1.000	.000	1.000	.000	.000	.979
C	.000	.000	.000	.000	.000	.000	.021
H	.000	.000	.000	.000	.000	.000	.041
H(unb)	.000	.000	.000	.000	.000	.000	.042
H(D.C.)	.000	.000	.000	.000	.000	.000	.042

Mean heterozygosity per locus (biased estimate) = .006 (S.E. .006)
Mean heterozygosity per locus (unbiased estimate) = .006 (S.E. .006)
Mean heterozygosity per locus (direct-count estimate) = .006 (S.E. .006)
Mean number of alleles per locus = 1.14 (S.E. .14)
Percentage of loci polymorphic (0.95 criterion) = .00
Percentage of loci polymorphic (0.99 criterion) = 14.29
Percentage of loci polymorphic (no criterion) = 14.29

Allele frequencies and genetic variability measures of electrophoretic data
species.

Population: SCARIOSUM (SC)

	Locus and sample size						
Allele	PGI-1 23	TPI-1 36	TPI-2 37	LAP-1 15	APH-1 23	AAT-1 22	PGM-1 36
A	1.000	.042	1.000	.000	1.000	1.000	.014
B	.000	.958	.000	1.000	.000	.000	.972
C	.000	.000	.000	.000	.000	.000	.014
H	.000	.080	.000	.000	.000	.000	.054
H(unb)	.000	.081	.000	.000	.000	.000	.055
H(D.C.)	.000	.083	.000	.000	.000	.000	.056

Mean heterozygosity per locus (biased estimate) = .019 (S.E. .013)
Mean heterozygosity per locus (unbiased estimate) = .019 (S.E. .013)
Mean heterozygosity per locus (direct-count estimate) = .020 (S.E. .013)
Mean number of alleles per locus = 1.43 (S.E. .30)
Percentage of loci polymorphic (0.95 criterion) = .00
Percentage of loci polymorphic (0.99 criterion) = 28.57
Percentage of loci polymorphic (no criterion) = 28.57

Population: HOOKERIANUM (HO)

	Locus and sample size						
Allele	PGI-1 44	TPI-1 58	TPI-2 40	LAP-1 35	APH-1 45	AAT-1 35	PGM-1 48
A	1.000	.043	.988	.000	1.000	1.000	.188
B	.000	.940	.013	.814	.000	.000	.813
C	.000	.017	.000	.186	.000	.000	.000
H	.000	.115	.025	.302	.000	.000	.305
H(unb)	.000	.116	.025	.307	.000	.000	.308
H(D.C.)	.000	.121	.025	.143	.000	.000	.167

Mean heterozygosity per locus (biased estimate) = .107 (S.E. .053)
Mean heterozygosity per locus (unbiased estimate) = .108 (S.E. .054)
Mean heterozygosity per locus (direct-count estimate) = .065 (S.E. .028)
Mean number of alleles per locus = 1.71 (S.E. .29)
Percentage of loci polymorphic (0.95 criterion) = 42.86
Percentage of loci polymorphic (0.99 criterion) = 57.14
Percentage of loci polymorphic (no criterion) = 57.14

Population: HOOKxLONG (X)

	Locus and sample size						
Allele	PGI-1 5	TPI-1 5	TPI-2 5	LAP-1 4	APH-1 5	AAT-1 5	PGM-1 5
A	.900	.000	1.000	.000	1.000	1.000	.000
B	.100	1.000	.000	.375	.000	.000	1.000
C	.000	.000	.000	.625	.000	.000	.000
H	.180	.000	.000	.469	.000	.000	.000
H(unb)	.200	.000	.000	.536	.000	.000	.000
H(D.C.)	.200	.000	.000	.250	.000	.000	.000

Mean heterozygosity per locus (biased estimate) = .093 (S.E. .068)
Mean heterozygosity per locus (unbiased estimate) = .105 (S.E. .077)
Mean heterozygosity per locus (direct-count estimate) = .064 (S.E. .042)
Mean number of alleles per locus = 1.29 (S.E. .18)
Percentage of loci polymorphic (0.95 criterion) = 28.57
Percentage of loci polymorphic (0.99 criterion) = 28.57
Percentage of loci polymorphic (no criterion) = 28.57

Population: LONGxHOOK (LX)

	Locus and sample size						
Allele	PGI-1 12	TPI-1 12	TPI-2 12	LAP-1 11	APH-1 9	AAT-1 11	PGM-1 11
A	.958	.083	1.000	.000	1.000	1.000	.000
B	.042	.917	.000	.545	.000	.000	1.000
C	.000	.000	.000	.409	.000	.000	.000
D	.000	.000	.000	.045	.000	.000	.000
H	.080	.153	.000	.533	.000	.000	.000
H(unb)	.083	.159	.000	.558	.000	.000	.000
H(D.C.)	.083	.167	.000	.727	.000	.000	.000

Mean heterozygosity per locus (biased estimate) = .109 (S.E. .074)
Mean heterozygosity per locus (unbiased estimate) = .114 (S.E. .078)
Mean heterozygosity per locus (direct-count estimate) = .140 (S.E. .101)
Mean number of alleles per locus = 1.57 (S.E. .30)
Percentage of loci polymorphic (0.95 criterion) = 28.57
Percentage of loci polymorphic (0.99 criterion) = 42.86
Percentage of loci polymorphic (no criterion) = 42.86

Population: LONGISTYUM (LO)

	Locus and sample size						
	PGI-1	TPI-1	TPI-2	LAP-1	APH-1	AAT-1	PGM-1
Allele	14	30	36	35	36	35	31
A	.964	.133	1.000	.014	1.000	1.000	.000
B	.036	.833	.000	.600	.000	.000	1.000
C	.000	.033	.000	.386	.000	.000	.000
H	.069	.287	.000	.491	.000	.000	.000
H(unb)	.071	.292	.000	.498	.000	.000	.000
H(D.C.)	.071	.300	.000	.314	.000	.000	.000

Mean heterozygosity per locus (biased estimate) = .121 (S.E. .073)
Mean heterozygosity per locus (unbiased estimate) = .123 (S.E. .074)
Mean heterozygosity per locus (direct-count estimate) = .098 (S.E. .055)
Mean number of alleles per locus = 1.71 (S.E. .36)
Percentage of loci polymorphic (0.95 criterion) = 28.57
Percentage of loci polymorphic (0.99 criterion) = 42.86
Percentage of loci polymorphic (no criterion) = 42.86

APPENDIX III

Summary of Principal Component Analysis.

Eigenvalues of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
PRIN1	8.95259	4.37428	0.241962	0.241962
PRIN2	4.57832	0.60420	0.123738	0.365700
PRIN3	3.97412	.	0.107409	0.473109

ID	PRIN1	PRIN2	PRIN3
L	5.32762	-1.42814	1.24829
L	5.66437	-0.81522	1.94642
L	5.30231	1.20822	1.36906
L	2.87641	0.70227	0.12751
L	2.26257	2.08623	-2.81516
L	1.03792	1.92393	-2.94642
H	-0.71977	-0.81299	-1.94595
H	0.75144	-2.35082	1.23620
H	-0.59977	-2.04879	1.19420
H	1.06330	-2.14517	1.23879
I	-2.59933	-1.17734	-1.86975
I	-2.56822	-0.95166	-0.12984
S	-2.59528	-1.90440	0.75579
S	-2.31521	-2.85768	0.06297
S	-3.16861	-2.21038	-1.15217
S	-1.47966	-0.54100	0.89198
T	-4.60801	4.94440	5.79246
U	-3.38484	1.32185	-1.18387
X	0.93016	2.94578	-2.14927
Y	-1.45439	1.25429	-0.53418
Z	0.27699	2.85662	-1.13705

H C. hookerianum (Southern Montana)
I C. hookerianum (Northern Montana & British Columbia)
L C. longistylum
S C. scariosum (Montana)
T C. scariosum (Nevada)
U C. scariosum (Oregon)
X HX
Y LX
Z X